SCIENTIFIC VALIDATION OF ANTIDIABETIC ACTIVITY OF MODIFIED MUCOADHESIVE THIOZOLIDINEDIONE DERIVATIVE

Dissertation submitted to

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MASTER OF PHARMACY

submitted by

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CHAPTER-I

1. INTRODUCTION

1.1. Diabetes Mellitus

Diabetes mellitus is one of the most common and challenging disease conditions of 21st century. WHO defined Diabetes mellitus as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbance in carbohydrate, fat and protein metabolism resulting from defects in insulin, its action or both. It is a chronic complex progressive and multifactorial disorder with life threatening micro and macrovacular complications. It is a major cause of morbidity and mortality. Prevalence of DM are about more than 150 million diabetics across the world and more than one fifth of them are Indians. International Diabetes Federation, India has been declared India as "Diabetic Capital of the World" at the recent Conference in Paris.[26]

Diabetes mellitus is complex, heterogeneous and polygenic metabolic disease where there will be an absolute lack of insulin, decreased sensitivity to insulin or both and which results in abnormal glucose homeostasis and subsequent hyperglycemia. Mutual interaction between genetic and environmental factors plays an important role in the pathogenesis of diabetes mellitus. DM has been characterized by a variety of causes such as obesity, abdominal adiposity, genetic, ethnicity etc.

1.2. BMI Related Diabetes Mellitus

Obesity and increased BMI have a great impact on diabetes. The association between increased BMI and weight gain and risk of Diabetes mellitus is significant among Asians. Waist circumference (WC) cut point for Indians for any cardio-metabolic risk factors is 87 cm for men and 82 cm for women whereas that of BMI is 23 kg/m2 in both sexes. It is found that the developing countries adopt the western life styles like decreased physical activity and over consumption of cheap, energy dense food for past 20 years and as a result the rate of obesity has tripled in developing countries. Such changes have a direct influence on the child health of the country; the prevalence of overweight ranges from 10 to 25%.[35]

1.3. Prevalence Of Diabetes Mellitus

Diabetes mellitus has a strong genomic association, genome-wide association studies has catalogued a number of gene that have an influence on DM (with modest odds ratio ranges between 1.2 to 1.5) and they include TCF7L2, HHEX, CDKAL1, SLC30A8 etc.

In India nearly 75% of the Type-II DM has first degree family history of diabetes indicating a strong familial aggregation. Prevalence of insulin resistance is found to be high in Asian Indians and they need higher amount of insulin to maintain normoglycemia, Comparison of Asian Indians, Europeans and other ethic groups have shown that the former higher insulin response than others, at fasting and in response to glucose. Asian Indians also have some factors which decreases the insulin sensitivity such as central obesity and high percentage of body fat in comparison to many other populations.[30]

The incidence of diabetes is growing rapidly both in the United States and worldwide. For example, it is estimated that more than 180 million peoples are affected with diabetes, and also prevalence will be expected to more than double by the year of 2030. In the United States, approximately 21 million peoples are estimated to suffer from diabetes, and it is also a major cause of morbidity and mortality. Diabetes is heterogeneous group of syndrome characterized by an elevation of blood glucose caused by a relative or absolute deficiency of insulin. The American Diabetes Association (ADA) recognizes four clinical classifications of diabetes: Type-I diabetes (formerly insulin-dependent diabetes mellitus), Type-II diabetes (formerly non-insulin dependent diabetes mellitus), gestational diabetes, and diabetes due to other causes (e.g., genetic defects or medication induced). Gestational diabetes is defined as carbohydrate intolerance with onset or first recognition during pregnancy. It is important to maintain adequate glucose control during pregnancy, because uncontrolled gestational diabetes can lead to fetal macrosomia (overly large body) and shoulder dystocia (difficult delivery), as well as neonatal hypoglycemia.

1.4. Types of Diabetes

Based upon the etiology, diabetes mellitus can be divided into two main types

Type- I- Insulin dependent diabetes mellitus (Juvenile DM)

Type -II- Non- Insulin dependent diabetes mellitus(Adult type)

Type –I- occurs in childhood, mainly due to destruction of pancreatic beta cell islets through auto-immune mediated, resulting in absolute insulin deficiency.

Type –II is associated with an adulthood and elderly people, which are mainly due to insulin resistance or abnormal insulin secretion.[26]

1.4.1. Type-I- Diabetes Mellitus

The disease is characterized by an absolute deficiency of insulin caused by massive β-cell necrosis or loss of β-cell function is usually ascribed to autoimmune-mediated processes directed against β cell, and it may be triggered by an invasion of viruses or the action of chemical toxins which are involved destruction of β-cells, the pancreas fails to respond to glucose due to lipolysis, proteolysis, and glycogenolysis. Type 1 diabetes mellitus have shown classic symptoms like polydipsia, polyphagia, polyuria, and weight loss. The development and progression of neuropathy, nephropathy, and retinopathy are directly related to the extent of glycemic control (measured as blood levels of glucose and/or hemoglobin A_{1c} [HbA_{1c}]. Treatment purpose nowadays using required quantity of exogenous insulin administered subcutaneous route to avoid the catabolic state that results from and is characterized by hyperglycemia and life-threatening ketoacidosis. Amylin is a hormone that is co secreted with insulin from pancreatic β-cells following food intake. Pramlintide [PRAM-len-tide] a synthetic analog of amylin, may be used as an adjunct to insulin therapy. Other methods of insulin delivery, such as transdermal, buccal, and intranasal are currently under research progress.

1.4.2. Type –II Diabetes Mellitus

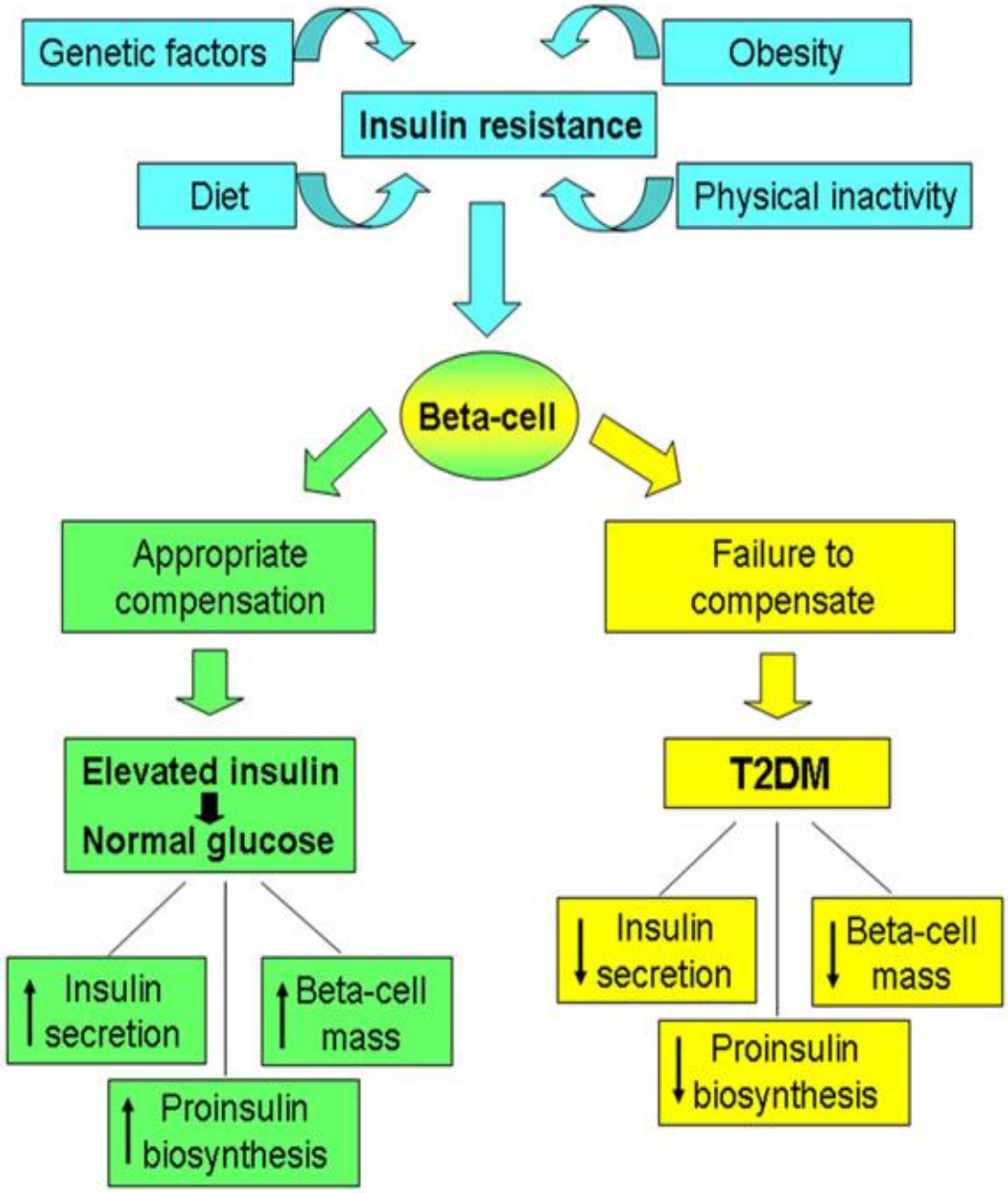
Type-II Diabetes Mellitus is multifactorial dysfunction of pancreatic β-cells due to influenced by genetic factors, ageing, obesity, and peripheral insulin resistance rather than by autoimmune processes or viruses. The goal for treating Type-I diabetes

mellitus is to maintain blood glucose concentrations within normal limits and to prevent the development of long-term complications of the disease. Weight reduction, exercise, and dietary modification decrease insulin resistance and correct the hyperglycemia of Type-II diabetes in some patients. However, most patients are dependent on pharmacologic intervention with oral hypoglycemic agents. As the disease progresses β- cell function declines, and insulin therapy is often required to achieve satisfactory serum glucose levels.

1.5. Causes of Diabetes[41]

Causes of diabetes depends on the type of diabetes. Type-I DM occurs mainly due to beta cell destruction, mediated through either immune mediated or idiopathic, whereas type-II diabetes occurs mainly due to insulin resistance or with relative insulin deficiency. Diabetes is also associated with life style factors and genetics. Other e are various types of other factors that involved in the development of diabetes which are the genetic material such as chromosomal and mitochondrial DNA mutation. Sometimes drugs and chemicals such as pentamidine, nicotic acid, glucocorticoids, thyroid hormones, beta adrenergic agonists, thiazides, alpha interferon can cause diabetes mellitus.abnormalities in the pancreas such as pancreatitis,pancresctectomy, neoplasia, cystic fibrosis fibrocalculous pancreatopathy can develop diabetes.

Figure No:1: Pro-Insulin Biosynthesis



1.6. Pathophysiology of Diabetes Mellitus[39]

Diabetes mellitus has a profound adverse effect on quality of life in terms of social, psychological well-being as well as physical health. Diabetic complications are mainly mediated through oxidative stress such as increased production of Reactive

Oxygen Species (ROS) or impaired antioxidant defence system. Enhancement of lipidperoxidation, alteration in antioxidant enzymes and impaired glutathione metabolism are the main factors involved in the development of diabetes. Production of free radicals are involved in the pathogenesis of various type of disease including diabetes mellitus. Increased formation and accumulation of advanced glycation products elimination (AGEs) also involved in the diabetic complications, such as retinopathy, neuropathy, nephropathy and renal dysfunction through a series of pathological changes. The several hormones are involved in the regulation of blood glucose level, the most importat alteration between insulin and glucagon. When imbalanced occurs in the level of hormones in the body, sugar starts accumulating in the blood when concentration of glucose increased in the blood then finally it will passes in urine along with other minerals. In most cases of diabetes, T-cell mediated action based 90% of the pancreatic islet beta cell destruction and also serological markers such as islet cell secreting enzymes are glutamic acid decorboxylase (GAD) are involved in the beta cells destruction.

1.7. Mucoadhesive Polymers Properties[3]

1. It must be loaded substantially by the active compound.
2. Swell in the aqueous and acidic biological environment of the delivery–absorption site.
3. Interact with mucus or its components for adequate adhesion.
4. Swelled, they allow controlled release of the active compound.
5. Excreted unaltered or biologically degraded to inactive, non-toxic oligomers.
6. Sufficient quantities of hydrogen bondingwith each chemical groups.
7. Possess high molecular weight.
8. Selective polymer has high chain flexibility.
9. It creates suface tension will induce penetration into mucous layer

1.8. Polymers In Mucosal Drug Delivery[7]

Bioadhesive polymeric systems have been used since long time in the development of products for various biomedical applications which include denture adhesives and surgical glue. After a lot of research, the researchers are of the view that a polymer will exhibit sufficient Mucoadhesive property if it can form strong intermolecular hydrogen bonding with the mucosal layer, penetration of the polymer into the mucus network or tissue crevices, easy wetting of mucosal layer and high molecular weight of the polymer chain. Polymers used in mucosal delivery system may be of natural or synthetic origin. In this section we will briefly discuss some of the common classes of mucoadhesive polymers.

1.8.1. Hydrophilic Polymers

The polymers are soluble in water. Matrices developed with these polymers swell when put into an aqueous media with subsequent dissolution of the matrix.[4] The polyelectrolytes extend greater mucoadhesive property when compared with neutral polymers. Anionic polyelectrolytes, Eg: poly (acrylic acid) and carboxy methyl cellulose, have been extensively used for designing mucoadhesive delivery systems due to their ability to exhibit strong hydrogen bonding with the mucin present in the mucosal layer**.** Chitosan provides an excellent example of cationic polyelectrolyte, which has been extensively used for developing mucoadhesive polymer due to its good biocompatibility and biodegradable properties. Non-ionic polymers,Eg:poloxamer, hydroxyl propyl methylcellulose, methyl cellulose, poly (vinyl alcohol) and poly (vinyl pyrrolidone),have also been used for mucoadhesive properties. The hydrophilic polymers form viscous solutions when dissolved in water and hence may also be used as viscosity modifying enhancing agents in the development of liquid ocular delivery systems so as to increase the bioavailability of the active agents by reducing the drainage of the administered formulations. These polymers may be directly compressed in the presence of drugs which are compressed form of mucoadhesive delivery system.

1.8.2. Hydrogels

Hydrogels defined as three-dimensionally crosslinked polymer chains which have the ability to hold water within its porous structure. The water holding capacity of the hydrogels is mainly due to the presence of hydrophilic functional groups like hydroxyl, amino and carboxyl groups. Hydrogels are prepared by the condensation reaction of poly (acrylic acid) and sucrose indicated an increase in the mucoadhesive property due to cross linking density and was attributed to increase in the poly (acrylic acid) chain density per unitarea. Acrylates have been used to develop Mucoadhesive delivery systems which has the ability to deliver peptide bioactive agents to the upper small intestine region without any change in the bioactivity of peptides. In addition to the drug targeting, Mucoadhesive hydrogel based formulations for improving the bioavailability of the poorly water soluble drug. This was attributed to the increased retention time of the delivery system within the gastrointestinal tract.[5]

1.8.3. Thiolated Polymers

The presence of free thiol groups in the polymeric skeleton helps in the formation of disulphide bonds with that of the cysteine-rich sub-domains present in mucin which can substantially improve the mucoadhesive properties of the polymers (e.g. poly (acrylic acid) and chitosan) in addition to the paracellular uptake of the bioactive agents.

1.8.4. Lectin-Based Polymers

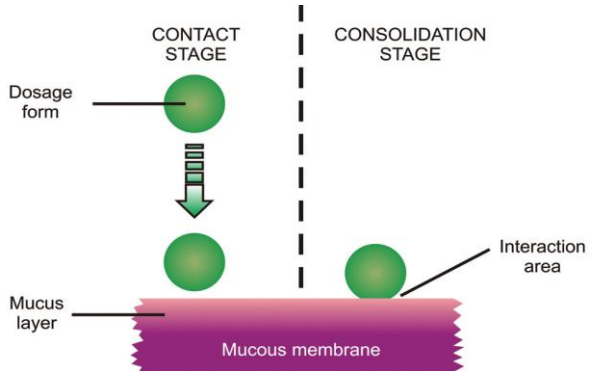
Lectins are proteins which have the ability to reversibly bind with specific sugar/carbohydrate residues and are found in both animal and plant kingdom and various microorganisms. The specific affinity of lectins towards sugar or carbohydrate residues provides them with specific cyto-adhesive property and it is being explored to develop targeted delivery systems. Lectins extracted from legumes have been widely explored for targeted delivery systems. The various lectins which have

shown specific binding to the mucosa include lectins extracted from *Ulex europaeus* , soybean, peanut and *Lens culinarius*.

1.9. Mechanism of Mucoadhesion

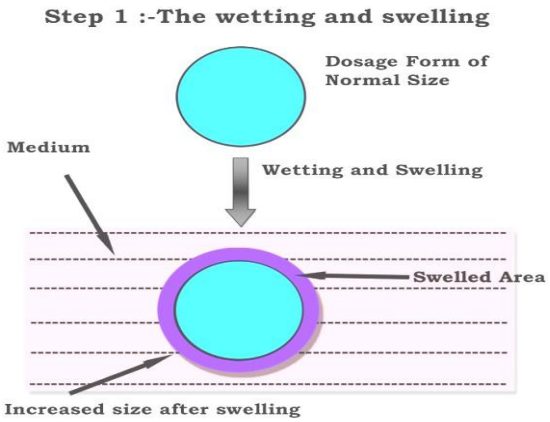
Thus, the mechanism of mucoadhesion is generally divided in two steps, the contact stage and the consolidation stage (Figure 2). The first stage is characterized by the contact between the mucoadhesive and the mucous membrane, with spreading and swelling (step 1) of the formulation. Essentially, there are two theories explaining the consolidation step: the diffusion theory (step 2) and the dehydration theory(step 3).

Figure. No: 2: The two steps of the mucoadhesion process



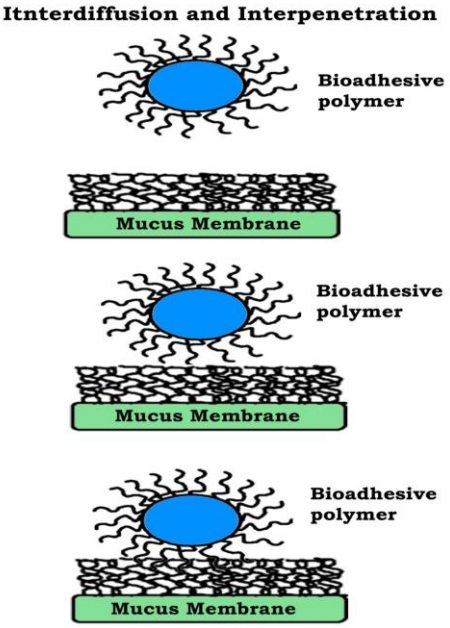
Step 1 :-The wetting and swelling step occurs when the polymer spreads over the surface of the biological substrate or mucosal membrane in order to develop an intimate contact with the substrate Bioadhesives are able to adhere to biological membrane or bond with biological tissues due to alteration of surface tension and forces that exist at the site of adsorption or contact. Swelling of polymers occur because the components within the polymers have an affinity for water.[12]

Figure. No: 3: Wetting and Swelling of Polymer



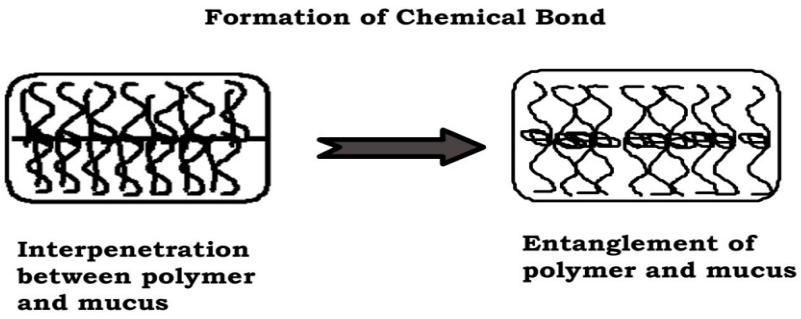
Step 2 : The surface of mucosal membranes are composed of high molecular weight polymers known as glycoproteins. In this step interdiffusion and interpenetration takes place between the chains of mucoadhesive polymers and the mucous gel network creating a great area of contact. The strength of these bond depends on the degree of penetration between the two polymer groups. In order to form strong adhesive bonds, one polymer group must be soluble in the other and both polymer types must be of similar chemical structure.

Figure. No: 4: Wetting and Swelling of Polymer (Membrane)



Step 3:- In this step entanglement and formation of weak chemical bonds as well as secondary bonds between the polymer chains mucin molecule. The types of bonding formed between the chains include primary bonds such as covalent bonds, Van der waals interactions and hydrogen bonds. Both primary and secondary bonds are exploited in the manufacture of bioadhesive formulations which has strong adhesions between polymers are formed.

Figure. No: 5: Entanglement of Polymer and Mucus by Chemical bonds



1.10. Advantages Of Mucoadhesives:[11]

- A prolonged residence time at the site of drug action or absorption.
- A localization of drug action of the delivery system at a given target site.
- An increase in the drug concentration gradient due to the intense contact of particles with the mucosa.
- Termination of therapy is easy (except gastrointestinal)
- Permits localization of drug to the oral cavity for a prolonged period of time.

1.11. Limitations

- Drug administration via the buccal mucosa has certain limitations
- Drugs, which irritate the oral mucosa, have a bitter or unpleasant taste, odour, cannot be administered by this route.
- Only drugs with small dose requirements can be administered.
- Only those drugs, which are absorbed by passive diffusion, can be administered by this route.

- Over hydration may lead to the formation of slippery surface and structural integrity of the formulation may get disrupted by the swelling and hydration of the bioadhesive polymers.

CHAPTER-II

2. LITERATURE REVIEW

Vishal Kadam, et al, (2013)[9] investigated development and evaluation of Mucoadhesive buccal tablets containing antihypertensive drugs, Labetalol Hydrochloride. The tablets were prepared by direct compression method ,12 formulations were developed using various concentrations of carbopol as primary polymer along with any of the secondary polymer viz HPMC,sodium alginate,xanthan gum,guargum.

Prasanth, et al, (2012)[10] has formulated and evaluated the Mucoadhesive buccal patches of aceclofenac using different polymers like HPMC, Carbopol 934,Polyvinyl alcohol, Poly Vinyl Pyrolidine K30, Eudragit L100.Effect of variable polymers which significantly influenced Mucoadhesive characteristics and *in vitro* drug release using 3^2 factorial design.

Prasanna, et al, (2012)[23] Formulated buccoadhesive films of glibenclamide buccal films by solvent casting technique by using different polymers such as HPMC 15 cps, carbopol and polyvinyl pyrrolidine. The films were evaluated for physiochemical characteristics, *in vitro* drug release and *ex vivo* buccoadhesive strength. The satisfactory results were obtained in all prepared formulation and based the result G14 [PMC (150mg), CP (20mg) and PVP (30mg)] was the best one compared to others. The drug release of all formulations in buccal mucosa was studied which showed good correlation was observed between *in vitro* and *in vivo* co relation, thus revealing the ability of the formulation to reproduce the *in vitro* release pattern through *in vivo*.

Afrasim moin, et al, (2010) [12] has studied a sustained release matrix tablets of Diltiazem hydrochloride using Karaya gum (K), Locust bean gum (LB), Hydroxy propyl methyl cellulose (H) by direct compression. Hardness, friability, *in-vitro* release, drug content, SEM, FTIR, DSC has been evaluated. It concluded that Karaya gum combination with Locust bean gum (1:2) showed synergistic effect in controlling Diltiazem release.

Madgulkar, et al, (2009)[13] has studied the development of buccal adhesive tablet with prolonged antifungal activity. The tablets were prepared by direct compression. carbopol 934P, hydroxypropylmethylcellulose K4M and poly vinyl pyrollidone were used as polymers. The tablets were evaluated for swelling index, mucoadhesive strength and *in vitro* drug release. The drug release and bioadhesion was dependent on type and relative amounts of the polymers. The release of the drug was slow and it prolonged miconazole action.

Akapa, et al, (2008)[14] has studied the patches containing Hydrochlorothiazide (HCTZ) formulated with Ethyl cellulose (EC) and Hydroxyl propyl metyl cellulose inter polymer complexes. Patches prepared by casting are evaluated for diameter, thickness, swelling behaviour buccoadhesive strength, drug content analysis and *in-vitro* release studies. Higuchi's analysis of the release mechanism indicated that the release of HCTZ from the patches formulated with 1:1 and 2:1 ratio's of EC and HPMC predominantly occurred by a diffusional process.

Emami, et al, (2008) [15] has studied a new buccoadhesive system for controlled release of Verapamil hydrochloride developed using Carbomer (CP), Hydroxy propyl methyl cellulose and Sodium carboxy metyl cellulose (NaCMC) by direct compression method. Thicknesses, weight variation, hardness, drug content uniformity, swelling, mucoadhesive strength, drug release are evaluated. The buccoadhesive containing 53% Cp and 13.3% HPMC showed suitable release kinetics. The release behavior was Non-Fickian controlled by a combination of diffusion and chain relaxation mechanisms and best fitted zero-order kinetics.

Venkataraju, et al, (2007)[16] have developed a controlled delivery system for Propranolol hydrochloride using the synergistic activity of Locust bean gum (LGB) and Xanthan gum (X) by wet granulation method. Prepared tablets were evaluated for hardness, friability, composition % and *in-vitro* release study. The XLBG formulation showed a required release rate with zero-order release kinetics due to the synergistic interaction of the two biopolymers that produced a strong and elastic gel in the presence of a ternary component control the drug release process.

Sathya Meonah, et al, (2011)[48] PharmacognosticalandHypoglycemic activity of different parts solanum nigrum linn palnt.The leaves and fruit of solanum nigrum have significant hypoglycaemic activity, the flavanoids present in the plant might be

an active components responsible for this activity. We progress on to isolate the active biocomponent responsible for the activity.

Coman, et al, (2011)[46] Plants and natural Compounds with Antidiabetic Action.Recently, more attention is being paid to the study of natural products as potential antidiabetics. This mini review of the current literature is structured into three main sections foucused on: (a) plant extracts, (b) plant biomolecules and (c) other molecules that have been used for their anitdiabetic effects. Potential molecular mechanisam of action are also discussed.

Gupta, et al, (2007)[25] Tetrabutylammoniumbromide mediated Knoevengel condensation in water: synthesis of cinnamic acids**.**A simple, mild and environment-friendly procedure has been developed for Knoevenagel condensation between aromatic aldehydes or ketones and malonic acid in the presence of tetrabutylammoniumbromide and K2CO3 under microwave irradiation in water, The products areobtained in excellent yields and are in a state of high purity.

Agrawal, et al, (2012)[28] Reviewed on Thiozolidinone and considered it as a biologically important active scaffold that possesses almost all type of biological activities. Successful introduction of ralitoline as a potent anti-convulsant, etozoline as a anthihypertensive, piogliazone as a hypoglycemic agent and thiozolidomycin activity against streptomyces species proved potential of thiozolidinone moiety. This diversity in the biological response profile has attracted the attention of many researches to explore this skeleton to its multiple potential against severa; activities. This review is complementary to earlier reviews and aims to review the work reported on various biological activities of thiozolidinone derivatives from year 2000 to the beginning of 2011. Data are presented for active compounds, some of which have passed the preclinical testing stage.

Ronan Roussed, et al, (2012)[27] Use of TZD was not associated with increased incidence of major cardiovascular events in patients with diabetes from this large registry. Older patients experienced an increased risk of CHF over the study interval. Limitations of this study include its observational design and thus unmeasured confounders cannot be excluded.

Shih-Ann Chen, et al, (2012) Demonstrated that TZDs had obvious protective effects on the development of AF diabetic patients. Drugs acting as ligands to the PPAR-y may be potential up stream therapies fo AF prevention.

Mueller, et al, (2012)[29] Thiozolidiones decrease the proinflammatory cytokines IL-6 and IL-8 in endometrial stromal cells via PPAR-y-independent mechanism. A better understanding of the anti-inflammatory action of this class of drugs may improve their safety and efficacy for endometriosis treatment.

Rosanna Maccari, et al, (2011)[30] Evaluated2-Thioxo-4-thiazolidinone derivatives were as aldose reductase inhabitors (ARIs) and most of them exhibited good or excellent in vitro efficacy. Out of the tested compounds, most N-unsubstituted analogues were found to posses inhibitory effects at low micromolar doses and two of them exhibited higher potency than sorbinil, used as a reference drug.

Rosaria Ottana, et al, (2011)[31] Explored a new set of suitably substituted compounds for more effective 5-arylidence-4-thiazolidinones as aldose reductase inhibitors, (4,5 and 8). Acetic acids 5, particularly 5a and 5h, proved to be interesting inhibitors of the enzyme as well as excellent antioxidant agents that are potentially able to counteract the oxidative stress associated with both diabetic complications as well as other pathologies. Molecular docking experiments supported SAR studies.

Changyou Zhou, et al, (2010)[45] Performed Systematic structure-activity relationship (SAR) studies of ascreening lead led to the discovery of series of thiozolidinediones (TZDs) as potent GPR40 agonists. Among them, compound C demonstrated an acute mechanism-based glucose-lowering in an intraperitoneal glucose tolerance test (IPGTT) in lean mice, while no effects were observed in GPR40 knock-out mice.

Ossama EI-Kabbani, et al, (2010)[41] Determined the structure of aldehyde reductase (ALRI) internary complex with the concenzyme NADPH and [5-(3-carboxymethoxylidene)-2, 4-dioxothiazolidin-3 yl] acetic acid (CMD), a potent inhibitor of aldose reducaste (ALR2), at 1.99 A resolution. Molecular modeling calculations and inhibitory activity measurements of CMD and and [5-(3-hydroxybenzylidene)-2, 4-dioxothiazolidin-3 yl] acetic acid (HMD) indicated that

stacking interactions with several conserved active site tryptophan residues and hydrogen-bonding interactions with none-conserved C- terminal residue Leu 300 in ALR2 (Pro301 in ALR1) contributed to inhibitor selectivity.

Nanjan, et al, (2010)[36] Designed somenovel glitazones based on the structure-activity relationships as possible PPAR-y agonists. The manually designed glitazones were synthesized by using the appropriate synthetic schemes and screened for their in vitro antihyperglycemic activity by estimating glucose uptake by rat hemi-diaphragm, both in the absence and in the presence of external insulin. Some of the gliazones exhibited good antihyperglycemic activity in presence of insulin.

Rosaria Ottana et al, (2009)[43] Identified effective low molecular weight ononphoshorus monoanionic inhibitors of PTPs and synthesized 4-[5-arylidene -4-oxo-2-phenyliminothiazolidin-3yl)methy1]- benzoic acids (4) and evaluated their inhibitory activity against human PTP1B and LMW-PTP enzymes. The introduction of a a-phenylimino moiety onto the 4-thiazolidinone ring was designed to enhance the inhibitor/enzyme affinity by means of further favourable interactions with residues of the active site and surrounding loops. Molecular modeling experiments inside the binding sites of both enzymes were performed.

Guorong Fana, et al, (2009)[34] Evaluate toxicity and toxicokinetics of MCC-555, a treatment candidate for type 2 diabetes, a novel thiazolidinedione which has comparatively high anti-diabetic efficacy in beagle dogs. During the treatment and recovery periods, the effects of the test agent on mortality, body weight, food consumption. Hematology, serum biochemistry urinalysis, electrocardiogram (ECG), organ weights, bone marrow and histopathology were examined. Metabolites and metabolic style of MCC-555 are to be approved.

Kim Henriksen, et al, (2009)[37] Performed a head-to-head comparison of equipotent glucose lowering concentrations of the partial PPARy agonist balaglitazone and the full agonist piog;itazone in male diet-induced obese rats, to investige effects on bone formation, fluid retention and fat accumulation. MR scans of body fat and water showed that all treatment groups increased their fat mass, whereas only the pioglitazone 30 group accumulated water. Pioglitazone treatment led to reduction of the bone formation marker osteocalcin, whereas balaglitazone treatment

did not affect it. Balaglitazone is a novel PAPRy agonist, which potently lowers glucose levels, while it neither affects fluid retention nor bone formation parameters.

Sriman Narayanan, et al, (2009)[39] Synthesized a series of novel dispiropyrrolidines by 1,3 dipolar cycloaddition reaction with 5-arylidene-1,3-thiazolidine-2,4-dione and 5-arylidence-4thioxo-1,3-thiazolidine-2-one derivatives as dipolarphiles. The Structure and stereochemistry of the cycloadduct have been established by single crystal X-ray structure and spectroscopic techniques. Molecular docking studies were performed on 1FM9 protein. The synthesized compounds were screened for their antidiabetic activity.

Klopper, et al, (2009)[41],Concluded that A375 (DRO) melanoma cell growth is inhibited by rexinoid and TZD treatment and this response id dependent on RXR and PPARy receptor expression. M14 (5-16) melanoma cell growth is inhibited by rexinoid and retinoid treatment, and this response is dependent on RXR expression. These findings may help guide molecular-based treatment strategies in melanoma and provide insight for mechanisms of resistance to nuclear receptor targeted therapies in certain cancers.

Venero, et al, (2008)[38] Recognized paradoxical respose of HDL-C to some PPAR ligands and suggest that clinicians be aware that resiglitazone with fenofibrate may reduce HDL-C level and consider alternative medications should a decrease in HDL-C occur.

Giuseppe Derosa, et al, (2008)[42],Observed No Change BMI, probably because rosiglitazone was added to metformin, that could mitigate the body increase of rosiglitazone. Rosiglitazone improved glycemic control and insulin resistance correlated parameters when added to intolerant metformin patients. These data suggest that rosiglitazone may be the drug of choice for the treatment of overweight and obese type 2 diabetic patients.

Marcin baranowski, et al, (2008)[31] Investigated the effects of two-week pioglitazone treatment (3 mg/kg/d) on lipid and carbohydrate metabolism in the heart of rats fed on a standard chow or on a high fat diet (HFD) for three weeks. High-fat feeding increased myocardial protein expression of all peroxisome proliferatoractivated receptor (PPAR) isoforms. The greatest response was, however,

noted in the case of PPARy. Surprisingly, administration of pioglitazone induced accumulation of free fatty acids (FFA) and diacylglycerol in the heart in both groups, despite concomitant reduction in plasma FFA concentration. Result suggest that thiazolidinediones improve cardic insulin sensitivity by mechanisms other than reduction in intramyocardial lipid content.

Rosanna Maccari, et al, (2008)[30] Reported a series of non-carboxylic acid containing 2,4-thiazolidinedione derivatives, analogues of previously synthesized carboxylic acids which we had found to be very active in vitro aldose reductase (ALR2) inhibitors. Although the replacement of the carboxylic group with the carboxamide or N-hydroxycarboxamide one decreased the in vitro ALR2 inhibitory effect, this led to the identification of mainly non-ionized derivatives with micromolar ALR2 affinity. The 5-arylidene moiety deeply influenced the activity of these 2, 4-thiazolidinediones.

Carroll et al, (2008)[41] Identified a novel protein, mitoNEET, which was later shown to regulate the oxidative capacity of the mitochondria. This identified an alternative target for the glitazones suggesting a possible new drug target for the treatment of neurodegenerative diseases. Molecular docking studies employing the reported crystal structure revealed five possible binding pockets on mitoNEET.

Alvarex, et al,(2008)[38] Demonstrated that a sunset of patients with T2DM experienced a paradoxic decrease in HDL-C when taking a fibrate TZD combination.

Dundar et al, (2008)[37] Prepared a series of flavonyl-2,4-thiazolidinediones by Knoevenagel reaction. The synthesized compounds were tested for their ability to inhibit rat kidney aldose reductase (AR) and for their insulinotropic activities in INS-1 cells.

Paola Vicini, et al, (2008)[41] Synthesized 2-Heteroarylimino-5-benzylidene-4-thiazolidinones, un-substituted or carrying hydroxyl,methoxy, nitro and chloro groups on the benezene ring and assyed in vitro for their antimicrobial activity against Gram positive and Gram negative bacteria, yeasts and mould.relationship of 33 analogues possessing the 2 heteroarylimino-4 thiazolidinone structure is analysed through QSAR models.

Oya Bozdag-Dundar, et al, (2008)[42] Prepared a new series of chromonyl-2, 4-thiazolidinediones by Knoevenagel reaction with substituted 3-formylchromones and unsubstituted (1) or substituted 2,4-thiazolidione (2). The synthesized compounds were tested for their ability to inhibit rat kidney AR by an in vitro spectrophotometric assay.

Subho mozumdar, et al, (2008)[40] Described a simple, efficient, stereoselective, one pot three component condensation reaction between thiazolidine-2,4-dione, aldehyde and amine derivatives using monodispersed, recyclable and inexpensive Cu nanoparticles for the synthesis of thiazolidine-2,4-dione derivantives in excellent yields and high purity.

Maccari, et al, (2006)[44] Synthesized and tested several 5-benzyl-2,4-thiazolidinediones (5-7) as in vitro aldose reductase (ALR2) inhibitors. Most of them, particularly N-unsubstituted 5-benzyl-2, 4dioxthiazolidin-3-yl) acetic acids 7, displayed moderate to high inhibitory activity levels. I(n detail, the insertion of an acetic chain on N-3 significantly enhanced ALR2 inhibitory potency, leading to acids 7 which proved to be the most effective among the tested compounds.

Aurelio Ortiza et al.,(2005)[45],Carried out a novel reaction between oxazolidinethione and bromoacetyl bromide to afford N-substituted 2,4-thiazolidinediones through an intramolecular nucleophilic substitution reaction.

Maharana, et al,(2012)[46] Assessment of anti hyperglycaemic and antioxidant potential of leaves of Solanum nigrum linn.in alloxan induced diabetes.The present study report clearly depicated that the Solanum nigrum extract endowed with hypoglycaemic and anti hyperglycaemic activity due to its possible action on pancreatic and extra pancreatic site of glucose and lipid metabolism as evidenced by insulinotropic and antioxidant properties of the extract.

CHAPTER-III

3. AIM & OBJECTIVE

Diabetes mellitus is a type of multifactorial disease due to less physical activity, improper food intake, hereditary and usage of drugs. Synthetic drugs can't completely cure the diabetes mellitus but it can mitigate the symptoms and also it can possess few adverse effects such as hypoglycemia, liver toxicity, kidney toxicity, neuropathy, retinopathy, and immunological disturbances. Based on the above reasons, we have undertaken to investigate the synthetic origin of thiozolidione derivative as synthesized and modified with the help of knoevenagel condensation reaction and evaluated by using *invivo* techniques such as hypoglycemic effects in rat sand Oral Glucose Tolerance Test in rats. Based on the results to prepare mucoadhesive patch attached with thiozolidine dione derivatives evaluated by using specific *invivo* animal model

CHAPTER-IV

4. PLAN OF WORK

1. Review of literatures
2. Synthesis of modified Thiozolidine dione
3. *Invivo* Antidiabetic study of modified thiozolidine dione
    - Normal animal
        - OGTT
        - Hypoglycemic effect
4. Formulation of mucoadhesive Thiozolidine dione tablets
    - Powder characteristics
    - Preparation of mucoadhesive tablets by direct compression
5. Physiochemical Evaluation
6. Streptozotocin induced diabetic study

CHAPTER-V

5. MATERIALS AND METHODS

List of Chemicals Used:

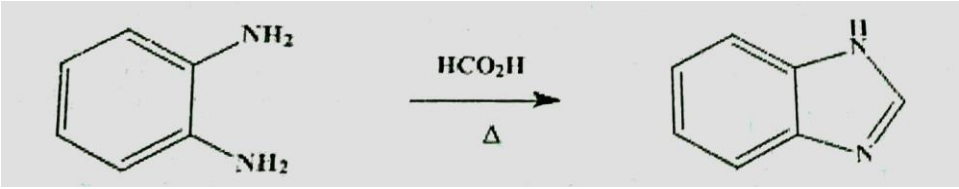
S. No	Chemicals	Company
1.	Aceclofenac	Micro labs
2.	Xanthan gum	Merck
3.	HPMC	Merck
4.	Guar gum	Merck
5.	Lactose	SD Fine chemicals
6.	Magnesium stearate	SD Fine chemicals

List of Equipment Used:

S. No	Equipments / Instrument	Company
1.	Digital balance	Shimadzu
2.	Digital pH meter	Mettler Toledo
3.	Hardness tester	Monsanto hardness tester
4.	Dissolution apparatus	Lab India
5.	U.V.Spectrophotometer	Shimadzu
6.	Bulk density apparatus	Mahalakshmi scientific
7.	Tablet Punching machine	Sakthi Multistation tablet press
8.	FTIR	Perkin Elmer

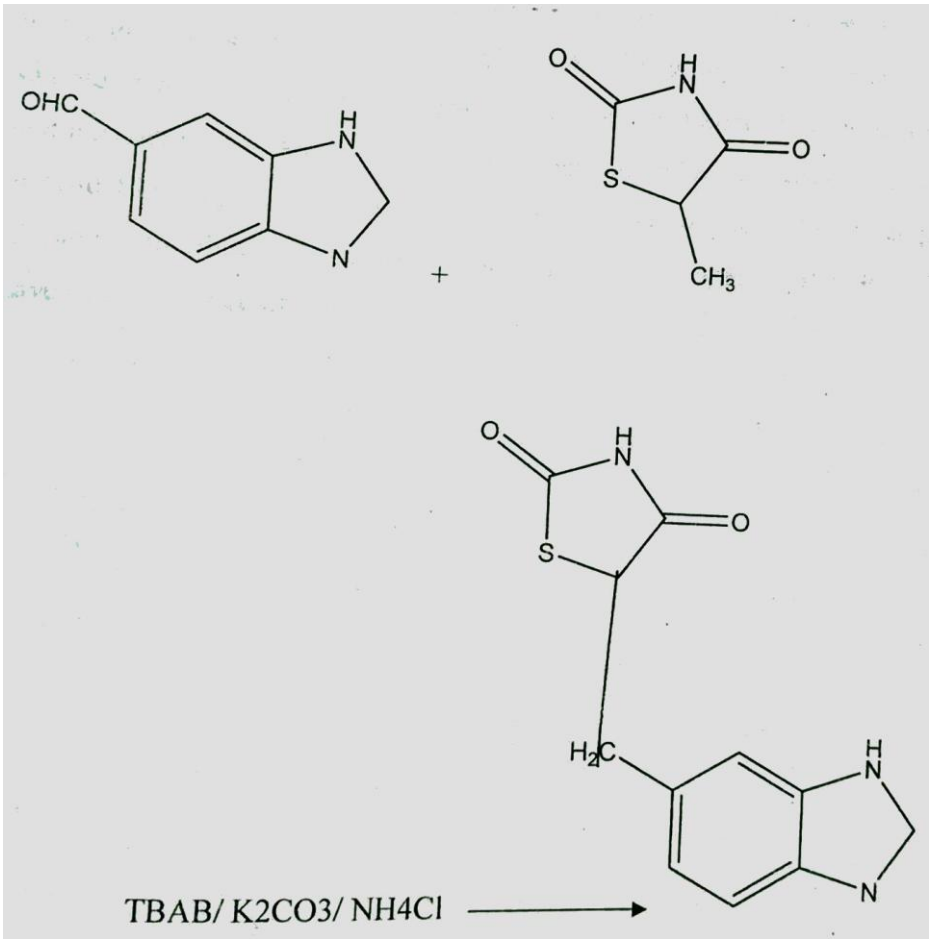
5.1. Synthesis of the Compound

In a 500-cc round-bound flask g(0.5mole) of O- Phenylenediamine is treated with 32 cc. (34.6g.) of 90 percent formic acid (0.75mole). The mixture is heated in a water bath at 100^{o}C for two hours. After cooling, 10 percent sodium hydroxide solution is added slowly, with through mixing by rotation of the flask, until the mixture is just alkaline to litmus. The crude benzimadazole is collected with suction in a 75-mm. Buchner funnel used for filtration under ice-cold water and to rinse all solid out of the reaction flask. The crude product is pressed thoroughly on the filter, washed with about 50 cc, of cold water, and then purified without previous drying.



5.2. Reaction between 2,4 thiozolidine and 4,formyl benzimidazole

Valizadeh et al, has reported the reaction between aldehyde and 2 methyl group of thiolidinedione using NH_4CL and water under MW irradiation but the products obtained are αβ-unsaturated melonic acid i.e. reaction stops before decarboxylation. Our developed method involves the irradiation of a mixture aromatic aldehyde or ketone with 2 methyl group using TBAB(Tetra Butyl Ammonium Bromide), K_2CO_3 and water. As soon as irradiation s stopped after few minutes of followed by acidification with dil.Hcl and separated out which are of high purity. When a mixture of benzaldehyde (5mmol), thiozolidinedione (5mmol), TBAB (2.5mmol), K_2CO_3(2.5mmol) and distilled water (10mL)was heated under microwaves for 5minutes at 900W, product was isolated as a pure product (TLC) in 85% yield.



Pharmacological Evaluation:

5.3. Animals

Wistar rats (150 – 250 g) used for the study were obtained from the animal house of the Department of Pharmacology, Karpagam College of Pharmacy, Coimbatore, Tamil Nadu. The animals are randomly selected, marked to permit individual identification and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were housed 6 per cage in a polypropylene food and water *ad libitum.*[18] All animal experiments were conducted in compliance with (Organization for Economic Cooperation and Development) OECD Guideline and approved by the Institutional Animal Ethics Committee, Karpagam University.

5.3.3. Drug administration

Animals are fasted prior to dosing (food but not water should be withheld for overnight). After that animals are weighed and the test substance administered. The healthy rats has been taken and divided into 4 different groups. The test substance was administered in a single dose by oral gavages, using a curved and ball tipped stainless steel feeding needle.

5.3.4. Experimental Design

In this study, 4 groups of 6 rats each were given with 5, 50 and 300 and 2000 mg/kg of the extract (p.o.) After drug administration the food is withheld for 3 hours. The animals are observed continuously for the first 2 hours, then occasionally up to 6 hours and then daily up to 14 days, post treatment to observe for any symptoms of toxicity and morality were carried out and changes were noted (OECD, 2001).

5.3.5. Clinical Observation

All animals were monitored continuously with special attention for 4 hr after dosing for signs of toxicity. Additional observations are also done for the next 14 days for any other behavioural or clinical signs of toxicity. Weight changes are calculated. At the end of the test animals are weighed. LD50 values are established using the formula.

Dose Calculation Equation

LD50 = higher dose - $\sum$ (a X b)/n

Where,

a = dose difference

b= animal died

n= No. of animals in each group

ED50=LD50

5.3.6. Selection of Test animals

Male wistar rats weighing 150 -200g were used for the present work. The animals used for the experiment were maintained under standard laboratory conditions in an animal house of Karpagam College of Pharmacy approved by the committee for the purpose of control and supervision on experiments on animals (IAECNO. KU/IAEC/ M. Pharm/169) under 12 h dark/light cycle and controlled temperature 24±2C. They had free access to food and water ad libitum. The animals were acclimatized to the laboratory for a period of 7 days, before the commencement of experiment.

5.3.7. Induction of Diabetes in Experimental Animals

Experimental diabetes was induced by single intra-peritonial injection of 25 mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer (pH 4.5) after 15 min of intra-peritonialinjection of nicotinamide (110 mg/kg) prepared in normal saline. Rats with marked glycosuria (Fasting blood glucose level greater than 200 mg/dL) after one week of administration of STZ were used for the study.

5.3.8. Assessment of diabetes

Diabetes was confirmed after 48 hr of streptozocin injection, the blood samples were collected through tail vein and plasma glucose levels were estimated by glucose oxidase method (accu check active glucometer). The rats having fasting plasma glucose levels more than 200 mg/dL were selected and used for the present study.

5.4. Oral Glucose Tolerance Test

The Oral Glucose Tolerance test (OGTT) measures the body's ability to use glucose, which is the body's main source of energy. Oral glucose tolerance test was performed in overnight fasted (18 hours) normal rats. Oral glucose tolerance test (OGTT) results have been expressed on table below. Half hour after the glucose treatment, all the groups of animal blood glucose levels were significantly increased. The blood glucose levels were significantly decreased when compared to control and positive control at 1hr and each and every ½ hour blood glucose levels were changes

in the dose dependent manner extract treated group of animals compared to control and positive control but 2mg/kg produce the equipotent activity.

5.5. Excipient Profile

5.3.1. Hydroxy Propyl Methyl Cellulose (HPMC)[18]

Synonyms	**:**	Benecel MPHC, Cellulose hydroxyl propyl methyl ether, E646, HPMC, Methocel, Metholose, Methyl cellulose propylene glycol ether, Methyl hydroxyl propyl cellulose, Pharmacoat.
Chemical name	**:**	Cellulose, 2-Hydroxy propyl methyl ether.
Empirical Formula	**:**	O-methylated & O- (2-hydroxy propylated cellulose)
Description	**:**	Odourless, tasteless, white or creamy white colored fibrous or granular.
Density	**:**	0.341 g/cm^3
Solubility	**:**	Soluble in cold water, forming a viscous colloidal solution, practically insoluble in chloroform, ethanol and ether.
Functional Category	**:**	Coating agent, film former, rate controlling polymer for sustained release, stabilizing agent, suspending agent, tablet binder, viscosity increasing agent..
Storage	**:**	Should be stored in well closes container in a cool, dry place.

5.3.2. Magnesium Stearate

Synonyms	**:**	Magnesium octadecanoate, Stearic acid magnesium salt, Octadecanoate acid, magnesium salt.
Chemical name	**:**	Octadecanoic acid magnesium salt
Empirical formula & Molecular Wt	**:**	$C_{36}H_{70}MgO_4$, 591.34
Structural formula	**:**	**[**$CH_3 (CH_2)_{16}COO$**]** $_2$ Mg
Density	**:**	0.159 g/cm^3
Solubility	**:**	Practically insoluble in ethanol, ether and water, slightly soluble in warm benzene.
Functional Category	**:**	Tablets and Capsule lubricant.
Storage	**:**	Should be stored in well closed container in a cool, dry place.

5.3.3. Guar Gum

Synonyms	**:**	Guar gum, Jaguar gum
Chemical name	**:**	Galactomannan polysaccharide
Empirical formula Molecular Wt	**:**	$(C_6H_{12}O_6)_n$, 220000
Structural Formula	**:**	It has linear chain of (1-4)-~~B~~-D-mannopyranosyl units

		with d-galactopyranosyl units
Solubility	**:**	Practically insoluble in ethanol, ether and water, slightly soluble in warm benzene.
Functional Category	**:**	Suspending agents, Tablet binders, Tablet disintegrant, Viscosity increasing agents.
Storage	**:**	Should be stored in well closed container in a cool, dry place.

5.3.4. Xanthan Gum[18]

Synonyms	**:**	Corn sugar gum, Rhodigel
Chemical name	**:**	Xanthan gum
Empirical formula & Molecular Wt	**:**	Dominant hexose units along with D-glucoronic acid,$2x10^6$
Structural Formula	**:**	5 Sugar residues: 2 glucose, 2 mannose and 1 glucoronic acid
Solubility	**:**	Practically insoluble in ethanol, ether and water, slightly soluble in warm benzene.
Functional Category	**:**	Stabilizing agents,Suspending agents, Viscosity increasing agents.
Storage	**:**	Should be stored in well closed container in a cool, dry place.

5.3.5. Lactose [18]

Synonyms	**:**	4(β-D-galactosido)-D-glucose, Lactochem, Microtose, Milk sugar, Pharmatose, Saccharum lactis, Tablettose, zeparox.
Chemical Name	**:**	O–β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose anhydrous O–β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate
Empirical formula & Molecular Wt	**:**	$C_{12}H_{22}O_{11}$ - 342.3(anhydrous),$C_{12}H_{22}O_{11}.H_2O$ – 360.31(monohydrate)
Description	**:**	White to off white crystalline particles or powder, odorless, slightly sweet – tasting
Density	**:**	1.552 g/cm^3
Hygroscopicity	**:**	It is stable in air and is unaffected by humidity at room temperature.
Solubility	**:**	Practically insoluble in chloroform, ethanol, ether, and slightly soluble in water
Functional category	**:**	Tablet and capsule diluents
Storage	**:**	Should be stored in well closed container in a cool, dry place.

5.6. Preparation of Mucoadhesive Tablets

The mucoadhesive drug/polymer mixture was prepared by homogeneously mixing the drug with Xanthan gum, HPMC, Guar gum, Lactose, Magnesium stearate.

The mixture of 150mg was then compressed using 6 mm diameter die in a single stroke multistation tablet machine.

Weighed all the ingredients and mixed thoroughly. Allow the mixture to pass thorough sieve no 60 & 80. Then added q.s isopropyl alcohol, make cohesive mass and passed through sieves no 20. Later dried the granules and separated through sieve no:35.

5.7. Evaluation of Mucoadhesive Tablets

The prepared mucoadhesive tablets were evaluated for following parameters.

5.7.1. Weight uniformity & Thickness

Initially individual weight of the tablets was calculated. The average weight of the formulated tablets was determined using electronic balance. Not more than two tablets should deviate from average weight.

Weight variation = Initial wt – Average wt / Initial wt x 100

Thickness was measured using screw gauge at different positions and average was calculated.

5.7.2. Hardness

The hardness of the tablets of each batch was measured by hardness tester . The hardness was measured in terms of Kg/cm^2 and it is found that hardness value of the formulation is 4.

5.8. Evaluation of Mucoadhesive characteristics

a) *In-vitro* mucoadhesive strength[21]

The adhesive strength of the mucoadhesive tablets was measured on the "Modified Physical Balance Method". The sheep buccal membrane was used as the model mucosal membrane. The fresh sheep buccal mucosa was cut into pieces and washed with phosphate buffer pH 6.8. A piece of mucosa was tied in to glass slide which was moistened with phosphate buffer pH 6.8. The tablet was stuck to the lower side of another glass slide with adhesive. The both pans were balanced by adding an appropriate weight on the left hand pan. The glass slide with mucosa was placed with appropriate support, so that the tablet touches the mucosa. Previously weighed beaker was placed on the right hand pan and water (equivalent to weight) was added slowly to it until the tablet detach from the mucosal surface. The weight required to detach the tablet from the mucosal surface gave the adhesive strength. The experiment was performed in triplicate and average value was calculated.

Figure. No: 5: Mucoadhesive strength measurement device

b) *Ex-vivo* residence time[21]

The *ex-vivo* mucoadhesion time was studied after application of tablets on freshly cut sheep buccal mucosa. The fresh sheep buccal mucosa was tied on the glass slide; the mucoadhesive core side of tablet was wetted with 1 drop of phosphate buffer pH 6.8 and pasted to the sheep buccal mucosa by applying light force with a finger tip for 30 seconds. The glass slide was then put in the beaker containing 100ml of the phosphate buffer pH 6.8 maintained at $37^{o}C \pm 0.2^{o}C$ and stirring was applied slowly to stimulate the buccal cavity environment and it was supplied with aeration. The tablet adhesion was monitored for 7hrs. The time at which the tablet detaches from the sheep buccal mucosa was recorded as the mucoadhesion time.

Figure. No: 7: Measurement of *ex-vivo* residence time

c) Swelling index

The swelling properties of the tablets were evaluated by determination of % swelling. The tablets of each formulation were weighed individually (W1) and placed separately in petri-dishes containing 2% agar gel. At regular time intervals (30,1,2,3,4,5,6 hours) the tablets were removed from petri-dishes and wiped off to remove the excess of water by using filter paper and the swollen tablets were reweighed (W2).

% Swelling index = [(W2 – W1) / W1] x 100

d) *In-vitro* release

The drug release rate from mucoadhesive tablets was studied using the USP XXIII (type II) rotating paddle method dissolution test apparatus. The dissolution test was performed using 500 ml of phosphate buffer pH 6.8 at 37±0.5^{o}C with a rotation speed of 50 rpm.

The samples (5ml, at each time) were withdrawn at pre-determined time intervals (0.5,1.0,2.0,3.0,4.0,5.0,6.0hr) from a zone midway between the surface of dissolution medium and the top of rotation paddle not less than 1cm apart from the vessel wall and volumes were replaced with fresh medium in order to maintain the sink condition. The samples were filtered through whatman's filter paper and were analyzed by UV Spectrophotometry at **216**nm against the phosphate buffer pH 6.8 as blank. The experiments for different formulations (F1-F6) were conducted in triplicate and average values were recorded.

e) *In-vitro* mucoadhesive permeation studies

The *in-vitro* Mucoadhesive drug permeation study of modified thiozolidine dione through the sheep buccal mucosa was performed using conventional permeation apparatus. The fresh buccal mucosa was tied to one end of open ended cylinder made up of glass, which acted as a donor compartment and the mucoadhesive tablets was placed with the core facing the mucosa. The donor compartment was filled with 10ml of phosphate buffer pH 6.8. The complete setup was then dipped into the receptor compartment containing phosphate buffer pH 6.8 which was maintained at 37^{o}C ±0.2^{o} C and hydrodynamics in the receptor compartment was maintained by stirring with a magnetic bead at a constant speed. The 5ml of samples were withdrawn at predetermined time intervals (0.5,1,2,3,4,5,6 &7 hours) filtered through a 0.2-µ filter and then the amount of Aceclofenac released was estimated by measuring the absorbance at **216**nm using UV spectrophotometer. The phosphate buffer pH 6.8 of same volume (5ml) pre warmed at 37^{o}C ± 0.2^{o}C was replaced to maintain its constant volume & sink condition. The cumulative amount of drug release was calculated and used while plotting the release and the release kinetics curves.

5.9. Streptozotocin Induced Diabetic Analysis

Streptozotocin or Streptozocin or lzostazin or zanosar (STZ) is a synthetic glucopyranose derivative isolated by the fermentations of Streptomyces achromogenes which posses anti-tumor antibiotic activity. It can be used to induce both type 1 and type 2 diabetes. Chemically it is (2-deoxy–2(3-methyl-3-nitrosoureido)-D-glucopyranose). The frequently used single i.v dose in adult rats to induce IDDM by immune system activation was found to be in between 40 and 60mg/kg. NIDDM can also be induced in rats by intravenous or intraperitoneal treatment with 100mg/kg b.w. STZ on the day of birth.[49]

STZ decrease insulin biosynthesis and secretion by impairing glucose oxidation. STZ at first abolished the B cell response to glucose. Temporary return of responsiveness than appears which is followed by its permanent loss and cells are damaged. STZ is taken up by pancreatic B cells via glucose transporter mainly GLUT-2. Intracellular action of STZ causes in changes of DNA I pancreatic B cells compromising its fragmentation. Alkylation of DNA is the main reason for the STZ induced B cells death.

STZ inhibits the Krebs cycle and decreases oxygen consumption by mitochondria and strongly limit mitochondrial ATP production and cause depletion of this nucleotide in B cells. Augmented ATP dephosphorylation increases the supply of substrate for xanthine and enhances for uric the final product of ATP degradation.

STZ is a Nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells. Hence it produces DNA damage. STZ is however not a spontaneous nitric oxide donor. STZ was found to generate reactive oxygen species, which also contribute to DNA fragmentation. The formation of superoxide anions result from both STZ action on mitochondria and increased activity of xanthine oxidase.

STZ induced DNA damage activates poly ADP ribosylation leading to the depletion of cellular NAD+ and ATP content and thereby inhabition of insulin biosynthesis and secretion. Calcium, which may also induce necrosis, does not seen to play a significant role.

CHAPTER-VI

6. RESULTS AND DISCUSSION

6.1. Synthesis of thiozolidine dione

Theoretical yield = Molecular weight of product / Molecular weight of reactant × weight taken

= (560/222×1.15)

Theoretical yield is found to be 2.90g

Practical yield = 2.60g

% yield = Practical yield / Theoretical yield × 100

% yield is found to be 89.65%w/w

6.2. Hypoglycaemic Activity

The Hypoglycaemic activity test results indicates modified thiozolidine dione treated animals 2mg/kg, significantly has decreased blood glucose level when compared to control and positive control which are clarified in the Table No:1

Table. No: 1 : Hypoglycaemic Activity

Treatment	Dose mg/kg	0 min	0.5hr	1hr
Control- Carboxy Methyl Cellulose (CMC)	0.5%	68.00±2.429	68.17±2.587	71.83±2.372
Positive Control (Pioglitazone)	2	68.00±0.632	52.83±4.037**	32.83±1.515***
Formulated Drug	2	68.80±2.429	53.00±2.309**	34.17±1.138***

The glucose levels were analysed by using glucometer and each value is the mean ±standard value (n=each group consist of 6 animals)($P<0.05$)*,($P<0.001$)**&($P<0.0001$)*** as compared to control & positive group evaluated by one way, ANOVA followed by Dunnet 't' test.

Figure. No: 8: Hypoglycaemic Activity at 30mins

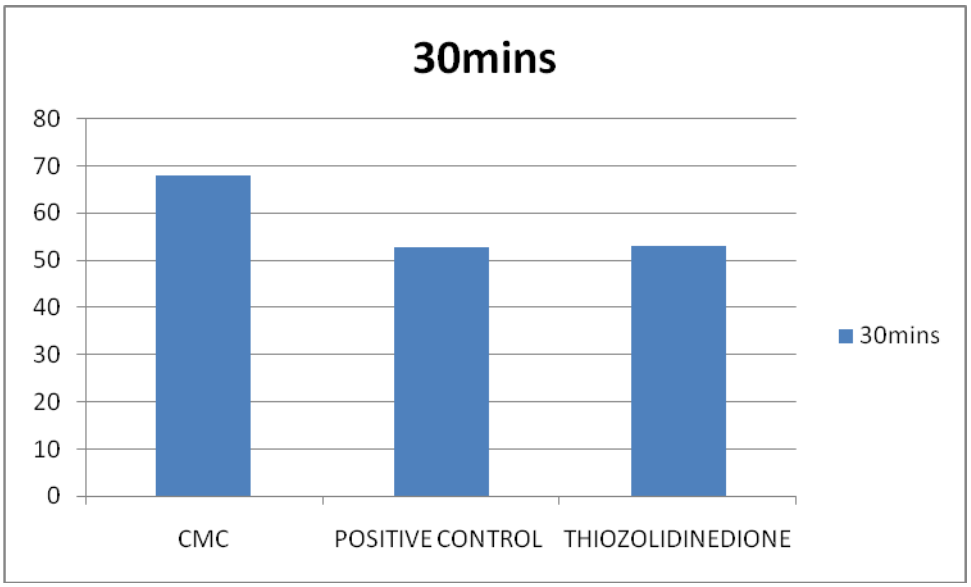
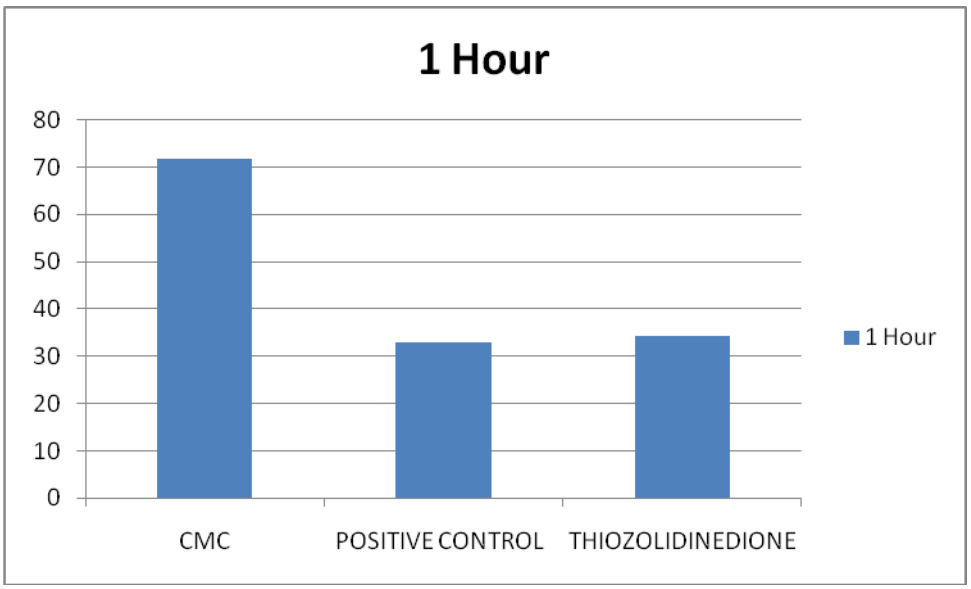


Figure. No: 9: Hypoglycaemic Activity at 1 hour



6.3. Oral Glucose Tolerance Test

Oral glucose tolerance test (OGTT) results have been expressed on table below. Half hour after the glucose treatment, all the groups of animal blood glucose levels were significantly increased. The blood glucose levels were significantly decreased

when compared to control and positive control at 1hr and each and every ½ hour blood glucose levels were changes in the formulation treated group of animals compared to control and positive control but 2mg/kg produce the equipotent anti diabetic action.

Table. No: 2: Oral Glucose Tolerance Test

Treatment	Dose mg/kg	0 min	0.5hr	1hr
Control- Carboxy Methyl Cellulose (CMC)	0.5%	68.00±2.429	142.5±6.292	187.5±9.465
Positive Control (Pioglitazone)	2	69.00±0.6325	104.2±7.323**	110.5±6.980***
Formulated Drug	2	68.80±2.245	128.3±6.009	147.3±2.404*

The glucose levels were analysed by using glucometer and each value is expressed as Means ± SEM (n=6))(P<0.05)*,(P<0.001)**&(P<0.0001)*** evaluated by one way, ANOVA followed by Dunnet 't' test.

Figure. No: 10: Oral Glucose Tolerance Test at 30 mins

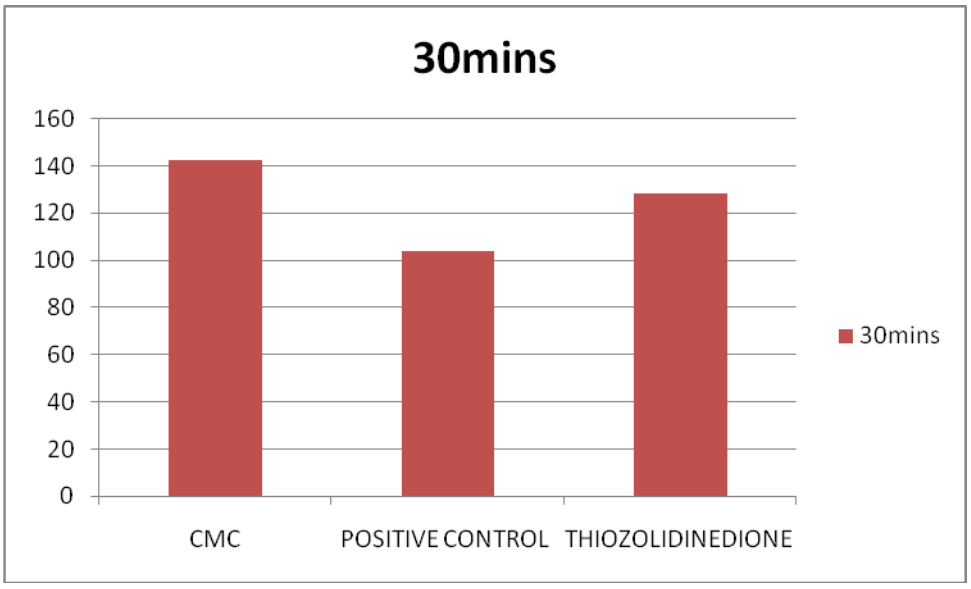
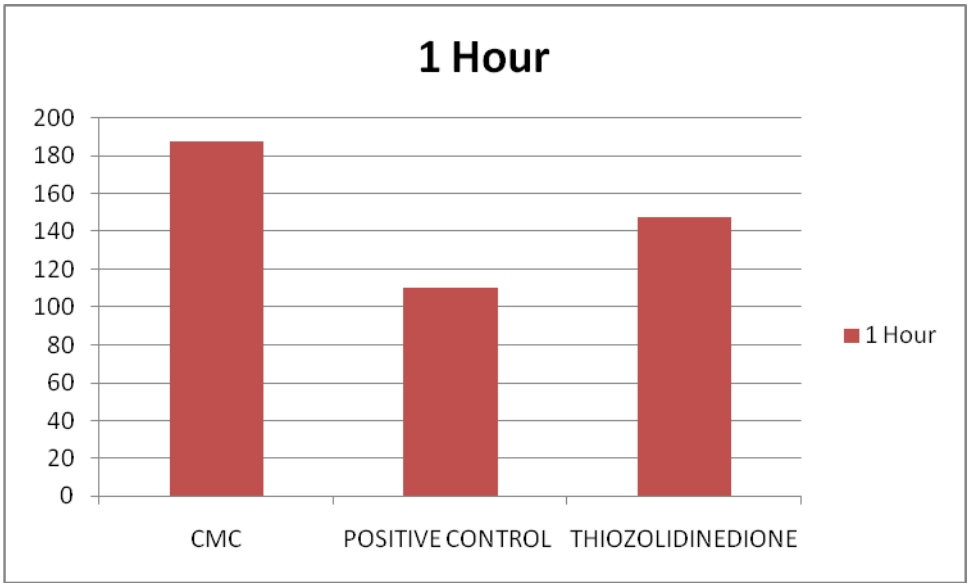


Figure. No: 11: Oral Glucose Tolerance Test at 1Hour



6.4. Evaluation of Mucoadhesive Tablets

Table. No: 3: Evaluation of Mucoadhesive Tablets

S.No.	Formulations	Evaluations		
		Weight Variation %	Thickness (mm)	Hardness (Kg/Cm^2)
1.	F1	2.0±0.3	2.4±0.05	4.2±0.12
2.	F2	2.4±0.8	2.43±0.03	4.0±0.20

6.5. *In-vitro* drug release profile of Formulations

Table. No: 3: Evaluation of Mucoadhesive Tablets

S.NO	Time (Hrs)	Formulations	
		F1	F2
1	0	0	0
2	0.15	47.5±0.6	16.25±1.5
3	0.30	62.5±0.4	20.0±0.9
4	1	88.0±1.2	37.5±1.0
5	2	-	81.25±0.9
6	3	-	90.0±1.3
7	4	-	-

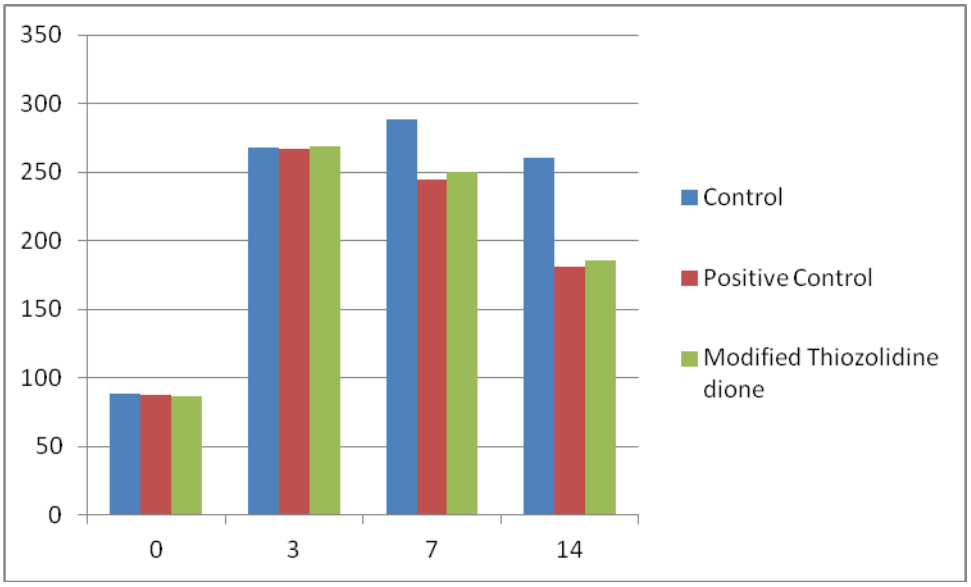
6.6. Streptozotocin induced diabetic evaluation

Table. No: 4: Treatment of modified thiozolidine dione tablet against STZ-induced diabetic rats (Glucose level mg / dl)

Sl. No	Treatment/ Dose (mg/ Kg)	Initial	3rd day	7th day	14th day
1.	Normal Control 0.5 % CMC	88.3±3.7	268.3±3.3	288.6±4.3	260.6±6.3
2.	Positive Control (Pioglitazone)-2	87.3±2.9	267.3±3.5	244.8±3.8***	180.8±4.2
3.	Formulated Drug-2	86.7±3.2	268.6±4.8	250±4.7**	185.7±6.8

Values are represented as Mean ± SEM (n=6 rats). Values are statistically significant at ** P< 0.01, ***P < 0.001. Diabetic + modified thiozolidinedione compared with diabetic + Pioglitazone and normal control rats.

Figure. No: 12: Treatment of modified thiozolidine dione tablet against STZ-induced diabetic rats (Glucose level mg / dl)



CHAPTER-VI
7. SUMMARY & CONCLUSION

The modified thiozolidine dione tablets have anti-diabetic activity, which are proved by the help of obtained results. In formulation aspect, mucoadhesive formulated modified thiozolidine dione has anti-diabetic activity which may be lesser toxicity. As per pharmacological aspect, in future to check toxicity of mucoadhesive formulated modified thiozolidine dione if it will be lesser toxicity so elucidate the exact structure of the modified thiozolidine dione with help of analytical studies to make pattern rights. This works will be useful for upcoming diabetic researchers to find new innovative formulation for the treatment of diabetes mellitus, which will be lesser adverse effects.

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